Canadian Journal of Microbiology
Revue canadienne de de microbiologie

Variability in colonization of arbuscular mycorrhizal fungi and its effect on mycorrhizal dependency of improved and unimproved soybean cultivars

Journal:	*Canadian Journal of Microbiology*
Manuscript ID	cjm-2016-0216
Manuscript Type:	Article
Date Submitted by the Author:	04-Apr-2016
Complete List of Authors:	Salloum, Maria Soraya; (a) Instituto Nacional de Tecnología Agropecuaria (INTA) Centro de Investigación Agropecuaria – (CIAP), Instituto de Fisiología y Recursos Genéticos Vegetales (IFRGV) Camino 60 Cuadras km. 51/2 Córdoba C.P. 5119. Argentina Guzzo, Maria Carla; (a) Instituto Nacional de Tecnología Agropecuaria (INTA) Centro de Investigación Agropecuaria – (CIAP), Instituto de Fisiología y Recursos Genéticos Vegetales (IFRGV) Camino 60 Cuadras km. 51/2 Córdoba C.P. 5119. Argentina Velazquez, Maria Silvana; Instituto Spegazzini, La Plata, Argentina Sagadin, Monica Beatriz; (a) Instituto Nacional de Tecnología Agropecuaria (INTA) Centro de Investigación Agropecuaria – (CIAP), Instituto de Fisiología y Recursos Genéticos Vegetales (IFRGV) Camino 60 Cuadras km. 51/2 Córdoba C.P. 5119. Argentina Luna, Celina Mercedes; (a) Instituto Nacional de Tecnología Agropecuaria (INTA) Centro de Investigación Agropecuaria – (CIAP), Instituto de Fisiología y Recursos Genéticos Vegetales (IFRGV) Camino 60 Cuadras km. 51/2 Córdoba C.P. 5119. Argentina
Keyword:	Arbuscular mycorrhizal fungi, Glomeromycota, oxidative stress, mycorrhizal dependency, improved and unimproved genotypes

Variability in colonization of arbuscular mycorrhizal fungi and its effect on mycorrhizal dependency of improved and unimproved soybean cultivars

M. S. Salloum[a], M.C. Guzzo[a], M. S. Velazquez[b], M. B. Sagadin[a], C.M. Luna[a]**

(a) Instituto Nacional de Tecnología Agropecuaria (INTA) Centro de Investigación Agropecuaria – (CIAP), Instituto de Fisiología y Recursos Genéticos Vegetales (IFRGV) Camino 60 Cuadras km. 51/2 Córdoba C.P. 5119. Argentina

(b) Instituto Spegazzini, La Plata, Argentina

****Corresponding author**

e-mail: luna.celina@inta.gob.ar

Telephone: 0054-0351-4973636

Fax: 0054-0351-4974330

Abstract

Variability in colonization of an AMF inoculum and in mycorrhizal dependency (MD) was compared between improved and unimproved soybean genotypes. The morphotaxonomic analysis of mixed native inoculum isolated from soybean roots, showed spores mostly of the species *Funneliformis mosseae*. Improved genotypes DM048 and NA09, named 1 and 2, respectively, and unimproved PI57440 and PI90768, named 3 and 4, respectively, were grown in a chamber under controlled conditions. While no variability in mycorrhizal colonization was observed in all genotypes at 20 days of inoculation, was evident the presence of arbuscules only in unimproved genotypes and improved genotype 2, but not in improved soybean genotype 1. At 40 days, mycorrhizal plants showed an increase in nodulation as compared with non-mycorrhizal ones, this effect was more evident in unimproved genotypes. Mycorrhizal dependency, evaluated as growth and biochemical parameters (chlorophyll, oxidative damage and antioxidant defenses) was significantly increased shortly in unimproved and improved genotype 2, whereas in genotype 1, MD increased at 40 days. These results show

variability in both mycorrhizal colonization and MD between unimproved and improved genotypes, suggesting that selection of soybean genotypes with good and rapid AM symbiosis could be a good strategy to improve yield in cropping systems.

Keywords: Arbuscular mycorrhizal fungi . (AMF) *Glomeromycota* . oxidative stress . mycorrhizal dependency·. improved and unimproved genotypes

Introduction

Inoculation with arbuscular mycorrhizal fungi (AMF; phylum *Glomeromycota*)) is an interesting strategy for improving crop yields because AM symbiosis provides numerous services to crops (Gianinazzi et al. 2010), including efficient use of fertilizers and soil nutrients (Javaid 2009), protection against drought stress (Porcel and Ruiz-Lozano 2004; Porcel et al. 2007) and diseases (Liu et al. 2007), increased N-fixation in legumes (Barea and Azcón-Aguilar 1983; Haselwandter and Bowen 1996), and improved soil physical properties (Hallett et al. 2009). Since AMF are obligate biotrophs that require a host plant to complete their life cycle (Smith and Read 2008), the effectiveness of AM symbiosis is highly dependent on the host plant genotype (Singh et al. 2012).

Different studies in crops indicate that there is genetic variability in AM colonization capacity among genotypes of host species (see Rengel 2002). Colonization by AMF of 13 wheat cultivars ranged from no infection to a high degree of infection (Azcon and Ocampo 1981). Specifically in soybean, Heckman and Angle (1987) reported variability in colonization in soybean roots by indigenous soil populations of AMF.

The concept of mycorrhizal dependency (MD) was defined by Plenchette et al. (1983) as the degree of plant growth change associated with AM colonization. Many investigations reported variability in MD, with plant growth being either positively or negatively affected (Klironomos 2003; Tawaraya 2003). In durum wheat genotypes, the degree of benefit from AM symbiosis was not proportional to the extent of root colonization (Al-Karaki and Al-Raddad 1997). Different species of important crop cultivars, such as *Glycine max, Vigna angularis, Senna tora, Hordeum vulgare, Zea mays, Sorghum bicolor, Allium tuberosum, Solanum melongena,* and *Capsicum annuum*, showed different responses to AMF inoculation (Eo and Eom

2009). More recently, Singh et al. (2012) tested five cultivars of modern durum wheat (*Triticum turgidum* L. var. durum Desf.) germplasm, and observed variations from low to high levels of AM root colonization, with no relationship being observed between wheat productivity and AM root colonization level.

Differences in MD were reported among wild, primitive and modern cultivated lines. Thus, MD showed variability in wheat of different ages (Kapulnik and Kushnir 1991; Zhu et al. 2001). The comparison of MD between modern *T. aestivum* cultivars and ancestors showed that cultivars released before 1950 had a higher MD than those released after 1950 (Hetrick et al. 1993), indicating that modern breeding programs might have reduced the responsiveness to AMF. More recently, Tawaraya (2003) reported mean MD values of 44% for field crops (37 species), 56% for forage crops (46 species), 70% for wild grasses and forbs (140 species), 79% for trees (26 species), and 56% for all plants (250 species), indicating that cultivated plant species showed a lower MD than wild ones. However, this correlation between cultivar age and mycorrhizal responsiveness is not universal, Khalil et al. (1994) found that, while some unimproved varieties of maize were unresponsive to mycorrhizal infection, others exhibited a 400% growth increase. Also Khalil et al. (1994) reported that AM colonization and MD of unimproved soybean *Glycine soja* cultivars was higher than those of improved cultivars.

In Argentina, despite the great economic importance of soybean crop, there is little information about its genotypic variability in mycorrhization. The objective of this study was to determine the existence of genetic variation in the compatibility of soybean plants with AMF. We compared unimproved and improved soybean cultivars, based on findings of variability in AMF colonization and MD reported by Khalil et al. (1994). Particularly, we tested a mixed native inoculum of AMF isolated from soybean roots. The development of the experimental system under controlled conditions allowed us to evaluate MD after short (20-day) and long (40-day) periods. Mycorrhizal dependency of soybean plants was determined by measuring growth and biochemical parameters related to oxidative stress regulation. We hypothesized that MD and mycorrhizal responsiveness to colonization of a mixed inoculum may differ between improved and unimproved soybean genotypes. The possible genetic variability among soybean cultivars might provide an opportunity to understand the mechanism underlying AM soybean colonization and to select for already colonized improved cultivars, which may be a useful strategy to manage soil AM resources.

Materials and methods

Plant and fungal material

The improved soybean genotypes used for this study were DM5048 and NA5009, characterized as susceptible and tolerant to drought stress, respectively, by Grumberg et al. (2015); hereafter, they are referred to as genotypes 1 and 2, respectively. Two wild soybean genotypes were also used, PI57440 (hereafter named genotype 3) and PI90768 (hereafter named genotype 4), belonging to INTA Marcos Juárez germplasm collection. The inoculum of AMF was obtained from soybean roots provided by EEA INTA Manfredi; the soybean crop was under a monoculture system. The inoculum was isolated and multiplied in pots using the plant-trap technique under greenhouse conditions, at 20-25 ºC, and watered daily with distilled water, for two years. After that period, the inoculum was propagated in pots containing soybean and *Medicago sativa* plants in a sterile sand/soil mix (1:1*v/v*).The spores were extracted from 100 gr of soil using different sieves (450-105-75-30 µm), according to Gerdemann and Nicolson (1963), and by centrifugation in sacarose gradient (Walker et al. 1982). The spores were identified under a light microscope using the morpho-taxonomic criteria of the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu/).

Plant fungus bioassays

The AMF inoculation experiment was set up in a completely randomized 4 x 2 factorial design, with one inoculation treatment and one water regime: well-watered conditions. Four genotypes were used and two inoculation treatments were performed: a mock-inoculation control (C = non-AM) and treatment with mixed native AMF inoculum.

Seeds of soybean genotypes were sterilized using 18% hypochlorite for 30 seconds. Then, two pregerminated seeds were introduced in pots containing a substrate consisting of sterile sand/soil mix (1:1). Seedlings were grown under controlled conditions in a chamber at 20–25 °C and watered daily with distilled water.

The mycorrhizal inoculum consisted of 8 g of soybean root fragments, spores and mycelia. Treated plants (hereafter referred to as AMF plants) were inoculated in the center of the pot; non-AMF plants treatments received the same amount of autoclaved inoculum. Before autoclaving, the inoculum was filtered with deionized water through a 37-µm sieve (Schleicher & Schuell, Germany). The filtrate was added to the non-AMF planting pots to provide them with the microbial populations accompanying the AMF, following Porcel and Ruiz Lozano (2004). The soil was watered with distilled water and maintained at normal moisture conditions until the end of the experiment. Samples of roots and aerial part were taken 20 and 40 days after treatment. The trials were repeated three times using 10 seedlings per genotype and per treatment.

Mycorrhizal dependency (MD) was calculated as [(M-NM)/M] x 100, using different growth parameters of individual mycorrhizal plants (M) and mean of different parameters corresponding non-AM plants (NM) (Plenchette et al. 1983). Plant biomass was measured as shoot plant height (SPH) and shoot dry mass (SDM), after drying to constant weight in oven at 70ºC. Leaf area (LA) was estimated from the first trifoliate leaves, by tracing the leaflet outlines on paper, cutting out the paper and weighing the cutouts; those weights were compared with the weight of a known area of paper (1 cm^2).

In addition, MD was calculated using different biochemical characters of oxidative stress that were evaluated in 100 mg of the second trifoliate soybean leaves. Oxidative damage was measured as lipid peroxidation, estimated as the content of 2-thiobarbituric acid-reactive substances and expressed as equivalents of malondialdehyde (MDA), according to Hodges et al. (1999). Total chlorophyll (TCh) was estimated by extracting the leaf material in 80% ethanol after incubating at 80 ºC for 15 min. Absorbance was recorded at 665, 645 and 470 nm and TCh was calculated according to Arnon (1949). Antioxidant defences were evaluated as FRAP assay (Benzie and Strain 1996). The AMF structures in the roots were stained according to Phillips and Hayman (1970) and colonization was measured following McGonigle et al. (1990).

Statistical analysis

Data of MD, calculated as [(M-NM)/M] x 100 and expressed as % relative to M treatments, were statistically analyzed using an analysis of variance (ANOVA). Differences among means were compared using DGC tests

($P \leq 0.05$). All statistical analyses were performed using the InfoStat Professional version 2013. No data transformation was required because MD percentage in morphological traits and biochemical parameters were normally distributed.

Results and discussion

Morpho-taxonomic characterization of native mycorrhizal inoculum

To our best knowledge, this is the first report on morphotaxonomic characterization of native mycorrhizal inoculum isolated from soybean roots. In mixed inoculum of AMF, *Funneliformis mossae* was the most abundant strain, with 427 spores per 100 g of soil, followed by *Paraglomus occultum* (147 spores), *Diversispora spurca* with 112 spores, *Glomus* sp. with 22 spores, and *Acaulospora scrobiculata* and *Gigaspora* sp., with only 7 and 3 spores, respectively (Fig. 1). *Funneliformis mossae*, the most abundant strain, corresponded to the taxonomic description of *Glomus mo*sseae and was also identified under a light microscope using the morphotaxonomic criteria of the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu/). Interestingly, *Funneliformis mosseae* is one of the most important AMF, being broadly distributed worldwide; recently, Al-Qarawi et al. (2013) indicated this AMF species as the most frequently present in rangeland ecosystems, particularly from dry soils of Saudi Arabia.

Variability of AMF colonization in improved and unimproved soybean genotypes

Our results show variability in mycorrhizal colonization of soybean genotypes, but such variability was dependent on inoculation time. During the short (20-day) test period (Table 1), percentage of soybean roots colonized by AMF was similar for all the genotypes. However, at 40 days of treatment, unimproved soybean genotypes exhibited the highest percentage of AMF root colonization, followed by improved soybean genotype 2, whereas no increase in mycorrhizal colonization was detected in improved soybean genotype 1 throughout the experiment. Accordingly, Heckman and Angle (1987) observed that cultivars of soybean *Glycine max* (L.) Merr were differentially colonized by indigenous soil populations of AMF. The cultivar colonized by the highest percentage was the nonnodulating isoline of Clark r*j*1 r*j*1 with 80% of root segments

examined being colonized, whereas 'Williams 82' exhibited the lowest percentage (57%) of AMF colonization. On the other hand, Khalil et al. (1994) reported percentages of unimproved and improved soybean roots colonized by AMF ranging from 62 to 87%, with the highest colonization being detected in *Glycine soja* (average 84%). Accordingly, we observed that unimproved soybean was more heavily colonized (64-72%) than improved soybean (38-42%) (Table 1, 40 days); these results are consistent with the idea that breeding selection of germplasm under fertilized conditions may reduce the frequency of genes that promote mycorrhizal associations (Linderman and Davis 2001; Ellouze et al. 2016).

Since our experimental system was developed under controlled conditions, we were able to identify mycorrhizal structures on two dates. Interestingly we observed variability in the arbuscule/hyphae ratio between soybean genotypes on the first observation date. Thus, at 20 days (Table 1), unimproved genotypes followed by improved genotype 2 had higher arbuscule content and higher arbuscule/hyphae ratio than improved genotype 1. Moreover, no arbuscule formation was detected in genotype 1on either date of observation. However, we observed the presence of coiled hyphae (data not shown), which was related to the control of nutrient transfer between symbionts without the presence of arbuscules (Smith et al. 2011).

Moreover, at 40 days of treatment, AMF-treated plants showed the presence of nodules. These results support previous findings showing that the number and weight of nodules increased significantly in AMF-colonized *Glycine max* (Varma 1979); and in *Medicago sativa* inoculated with *Glomus mossae* (Barea et al. 1980). It is known that flavonoids exuded by legume seedlings may not only be stimulants for hyphal growth of the AM fungi but also nod-gene inducers (see Rengel 2002). Furthermore, Spagnoletti et al. (2012) observed a high number of AMF spores and entry points of extermal mycelum inside nodules of alfalfa roots, showing a close association between AMF and nodules. Interestingly, a higher number and diameter of nodules was observed in unimproved soybean genotypes than in improved ones (Table 2). To our knowledge, this is the first report about these differences. The fact that unimproved genotypes exhibited the highest number and size of nodules suggests a greater N_2 biological fixing capacity than improved genotypes. Accordingly, Barea et al. (1980) reported an increase in N_2 biological fixation in *Medicago sativa* plants that showed an increase in AM symbiosis with the presence of rhizobacteria and nodules.

Mycorrhizal dependency in improved and unimproved soybean genotypes

Variablity in MD has been repeatedly reported in wheat after AMF colonization (Al-Karaki and Al-Raddad 1997; Kapulnik and Kushnir 1991; Singh et al. 2012). In particular, MD was higher in unimproved *Glycine soja* cultivars than in improved cultivars (Khalil et al. 1994). In our work, we evaluated soybean genotype symbiosis on two dates, which allowed us to detect MD variability in growth parameters in these genotypes. Thus, on the first date of observation of AMF-treated plants (20 days), unimproved genotypes 3 and 4, and improved genotype 2 showed a significant increase in plant biomass, expressed as MD in SPH, SDM and LA. By contrast, improved genotype 1 showed a negative MD in plant biomass parameters as compared to the other genotypes (Fig. 1). This behaviour changed at 40 days after treatment because improved genotype 1 showed a positive increase in MD, reaching, but not exceeding, similar MD values to those shown by the other genotypes after the short period (20 days) (Fig.1).

Mycorrhizal dependency was often measured as dry weight (Plenchette 1983; Hetrick et al. 1992); in the present study, MD was also evaluated via biochemical parameters related to oxidative stress and antioxidant defenses. Oxidative stress is defined as the increase in reactive oxygen species (ROS). Under normal conditions, ROS are produced mainly at a low level in organelles such as chloroplasts, mitochondria and peroxisomes. However, under biotic and abiotic stress, rate of ROS production is dramatically elevated; producing toxicity phenomena in plants, with the subsequent reduction in crop yield (Miller et al. 2010). Here, oxidative damage was evaluated as MDA, which is often regarded as the product and an indicator of the degree of membrane lipid peroxidation. In our study, MD in both improved 2 and unimproved genotypes 4 and 3 showed a negative MDA at 20 days, as compared to genotype 1 (Fig 5, 20 days). At 40 days, improved genotype 1 showed a negative percentage of MDA level, as the others soybean genotypes (Fig. 5, 40 days). Antioxidant defense was evaluated here as FRAP and TCh content. It is known that symbiosis with AMF stimulates chlorophyll synthesis (Auge et al. 2001), which has been related to an increase in photosynthetic metabolism in mycorrhyal plants (Gong et al. 2013). On the other hand, FRAP is an indirect measure of non-enzyme antioxidant content; therefore, we were interested in evaluating FRAP because key antioxidants, such as glutathione and ascorbic acid, have been related to antioxidant defense in AMF plants (Ruiz Lozano 2003). Again, after the short period of treatment, unimproved genotypes 3 and 4, and improved genotype 2 showed a higher MD content, measured as TCh and FRAP, than improved genotype 1 (Fig. 3). However, after the long treatment period (40 days), MD expressed as FRAP and TCh content increased significantly in genotype 1,

reaching similar values to those shown by the other genotypes after the short period (20 days). The fact that MD, evaluated as MDA content, showed negative values in all soybean genotypes suggests that oxidative damage was under control in inoculated plants. Many investigations agree in that mycorrhizal plants can reduce oxidative damage (Zhu et al. 2011); in particular, previous experiments conducted by our research group showed that after treatment with paraquat, oxidative stress, evaluated as MDA foliar content, decreased in soybean mycorrhizal plants as compared with non-mycorhizal plants (Bressano et al. 2010) and under drought stress (Grumberg et al. 2015).This effect was correlated with an increase in FRAP and TCh content, suggesting the participation of non-enzymatic antioxidant defences. Interestingly, our results show that unimproved genotypes 3 and 4 followed by improved soybean genotype 2 were able to regulate oxidative damage over a short period, 20 days of treatment, indicating that oxidative damage regulation is a phenomenon closely associated with AMF symbiosis, with this association being stronger in unimproved soybean genotypes than in improved ones.

Khalil et al. (1994; 1999) found that both MD and AM colonization increased in unimproved soybean genotypes, showing greater benefits from mycorrhizal symbiosis than modern cultivars. In our study, at the end of experiment and in agreement to Khalil et al. (1994; 1999), both unimproved genotypes exhibited a higher AM colonization as well as a higher arbuscule formation, an increase in nodules and a higher capacity of oxidative stress regulation than improved soybean genotype 1.

However, at early time of experiment (20-day), AM colonization percentage was similar in all genotypes, whereas responses to MD were different, with unimproved genotype 4 exhibiting the highest MD and improved genotype 1 showing the lowest MD level. Singh et al. (2012) indicated that MD alone is not an appropriate breeding target because the yield of a variety dependent on AMF may be vulnerable to conditions of inhibited or limited soil AM fungal resources. Accordingly, our results show that improved genotype 1 even increased MD at the end of assay, showing a slower and lower MD response after the short period than unimproved genotypes 3 and 4 and improved genotype 2. These genotypes had early responses to arbuscule formation and enhanced MD, suggesting that they could overcome the AM-deficient soils and still be productive, independently of AM symbiosis. In agreement with this idea, preliminary results found by our research group show that, after being exposed to early drought stress, both unimproved and improved genotype 2 had better capacity to tolerate stress than improved genotype 1 (data not shown). We propose that

the high presence of arbuscular structures in unimproved soybean cultivars could be related to their early increase in MD. The presence of arbuscules seems to be critical for simbiotic function and has been associated with increased metabolic activity in mycorrhizal plants, since arbuscules may facilitate bidirectional exchange of nutrients between plants and the fungus (Smith and Smith 2011). Recently, Park et al. (2015) demonstrated that a transcription factor, RAM1, regulates at least two genes required for enabling arbuscule branching and is related to different levels of arbuscular colonization and productive symbiosis. Also, Park et al. (2015) suggest that transcript levels of RAM1 are modulated by DELLA activity, providing information about the transcription factors through which DELLAs act to enable development of arbuscules. We suggest that the different ratio of arbuscule/hyphae between improved and unimproved soybean genotypes may be associated with different RAM1 activation.

Conclusion

Our findings suggest genetic variation in compatibility with mixed native of AMF inoculum between unimproved and improved soybean genotypes. Differences in MD between unimproved and improved soybean genotypes may help to elucidate the different mechanisms responsible for this compatibility and different physiological indices could be used as indicators of genetic improvement in mycorrhizal soybean genotypes. Moreover, selection of improved soybean genotypes with good and rapid AMF colonization and high arbuscule/hyphae ratio appears as a useful strategy for the development of varieties that optimize AMF contribution to cropping systems.

Acknowledgements This work was funded by National Promotion Agency Science and Technology (ANPCyT) through the Fund for Scientific Research and Technology (FONCyT) : Projects of Scientific and Technological Research (PICT) 2012-0339 and by the National Institute of Agricultural Technology (INTA) through Projects Nº1133032 and Nº1127033. The National Council of Scientific and Technical Research (CONICET) awarded the scholarship to M.S. Salloum.

References

Al-Qarawi, A, A., Mridha, M.A.U. and Dhar, P.P. 2013. Report of *Funneliformis mosseae* (Nicol and Gerd) Gerd and Trappe in Rangeland Soil of Saudi Arabia. Res J Bio Technology 8(2): 93-96.

Al-Karaki, G.N., and Al-Raddad, A.1997. Drought stress and VA mycorrhizal fungi effects on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Crop Res (Hisar) 13:245–257.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24:1–15.

Auge, R.M. 2001. Water relations, drought and VA mycorrhizal symbiosis. Mycorrhiza 11:3-42.

Azcon, R., and Ocampo, J.A. 1981. Factors affecting the vesicular arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytol. 87(4), 677-685.

Barea, J.M., Escudero, J.L., and De Aguilar, C.A.G. 1980. Effects of introduced and indigenous VA mycorrhizal fungi on nodulation, growth and nutrition of *Medicago sativa* in phosphate-fixing soils as affected by P fertilizers. Plant soil 54(2), 283-296.

Barea, J.M., and Azcón-Aguilar, C. 1983. Mycorrhizas and their significance in nodulating nitrogen-fixing plants. Adv Agron 36: 1–54. doi:10.1016/S0065-2113(08)60351-X.

Benzie, I.F., and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal biochem. 239(1), 70-76.

Bressano, M., Curetti, M., Vargas Gil, S., Cabello, M., March, G., Ducasse, D.A., and Luna, C.M. 2010. Arbuscular mycorrhizal fungi confer enhanced paraquat tolerance to soybean plants. J Plant Physiol. 167:1622-1626.

Ellouze, W., Hamel, C., DePauw, R.M., Knox, R.E., Cuthbert, R.D., Singh A. K. 2016. Potential to breed for mycorrhizal association in durum wheat. Can. J. Microbiol. 62, (3): 263-271 (doi: 10.1139/cjm-2014-0598).

Eo, J.K., and Eom, A.H. 2009. Differential growth response of various crop species to arbuscular mycorrhizal inoculation. Mycol. 37(1): 72-76.

Gerdemann, J.W., and Nicolson, T.H. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Brit Mycol Soc. 46:235-244.

297 Gianinazzi, S., Gollotte, A., Binet, M.N., Van Tuinen, D., Redecker, D., and Wipf, D. 2010. Agroecology: the
298 key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20(8): 519–530.
299 doi:10.1007/s00572-010-0333-3. PMID: 20697748.
300 Gong, M., Tang, M., Chen, H., Zhang, Q., and Feng, X. 2013. Effects of two Glomus species on the growth
301 and physiological performance of Sophora davidii seedlings under water stress. New Forest 44, 399-408.
302 Grümberg, B.C., Urcelay, C., Shroeder, M.A., Vargas-Gil, S., and Luna, C.M. 2015. The role of inoculum
303 identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean. Biol Fertil Soils 51(1), 1-
304 10.
305 Hallett, P.D., Feeney, D.S., Bengough, A.G., Rillig, M.C., Scrimgeour, C.M., and Young, I.M. 2009.
306 Disentangling the impact of AM fungi versus roots on soil structure and water transport. Plant Soil 314(1–
307 2): 183–196. doi:10.1007/s11104-008-9717.
308 Haselwandter, K., and Bowen, G.D. 1996. Mycorrhizal relations in trees for agroforestry and land
309 rehabilitation. Forest Ecol Manag. 81(1), 1-17.
310 Heckman, J.R., and Angle, J.S. 1987. Variation between soybean cultivars in vesicular-arbuscular mycorrhiza
311 fungi colonization. Agron J. 79(3), 428-430.
312 Hetrick, B.A.D., Wilson, I.G.W.T., and Cox, T.S. 1992. Mycorrhizal dependence of modern wheat cultivars
313 and ancestors: a synthesis. Can J Bot. 71: 512-518.
314 Hetrick, B.A.D., Wilson, G.W.T., and Cox, T.S. 1993. Mycorrhizal dependence of modern wheat cultivars
315 and ancestors: a synthesis. Can J Bot. 71(3): 512–518. doi:10.1139/b93-056.
316 Hodges, D.M., DeLong, J.M., Forne, C.F., and Prange, R.K. 1999. Improving the thiobarbituric acid-reactive-
317 substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other
318 interfering compounds. Planta 207: 604–611.
319 Javaid, A. 2009. Arbuscular mycorrhizal mediated nutrition in plants. J Plant Nutr 32(10): 1595–1618.
320 doi:10.1080/ 01904160903150875.
321 Kapulnik, Y., and Kushnir, U. 1991. Growth dependency of wild, primitive and modern cultivated wheat lines
322 on vesicular-arbuscular mycorrhiza fungi. Euphytica 56(1), 27-36.
323 Khalil, S., Loynachan, T.E., and Tabatabai, M.A. 1994. Mycorrhizal dependency and nutrient uptake by
324 improved and unimproved corn and soybean cultivars. Agron J. 86: 949–958.

Khalil, S., Loynachan, T.E., and Tabatabai, M.A. 1999. Plant determinants of mycorrhizal dependency in soybean. Agron J. 91(1), 135-141.

Klironomos, J.N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecol. 84(9), 2292-2301.

Liu, J., Maldonado-Mendoza, I., Lopez-Meyer, M., Cheung, F., Town, C.D., and Harrison, M.J. 2007. Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50(3): 529–544. doi:10.1111/j.1365-313X.2007.03069.x. PMID:17419842.

Linderman, R.G., and Davis, E.A. 2001. Comparative response of selected grapevine rootstocks and cultivars to inoculation with different VA mycorrhizal fungi. Am. J. Enol. Viticult. 52, 8–12.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., and Swan, J.A. 1990. A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495–501.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., and Mittler, R. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 33:453–467. doi:10.1111/j.1365-3040.2009.02041.x.

Park, H.J., Floss, D.S., Levesque-Tremblay, V., Bravo, A., and Harrison, M.J. 2015. Hyphal branching during arbuscule development requires RAM1. J Plant Physiol. pp-01155.

Phillips, J.M., and Hayman, D.S. 1970. Improved procedure of clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 55:159–161.

Plenchette, C., Fortin, J.A., and Furlan, V. 1983. Growth responses of severalplant species to mycorrhizae in a soil of moderate P-fertility. I Mycorrhizal dependency under field conditions. Plant Soil 70:199–209.

Ruiz-Lozano, J.M. 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies. Mycorrhiza 13, 309–317.

Porcel, R., and Ruiz-Lozano, J.M. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot. 55: 1743-1750.

352 Porcel, R., Aroca, R., Cano, C., Bago, A., and Ruiz-Lozano, J.M. 2007. A gene from the arbuscular
353 mycorrhizal fungus Glomus intraradices encoding a binding protein is up-regulated by drought stress in
354 some mycorrhizal plants. Environ Exp Bot 60(2): 251– 256. doi:10.1016/j.envexpbot.2006.11.001.
355 Rengel, Z. 2002. Breeding for better symbiosis. Plant Soil 245: 147–16.
356 Singh, A.K., Hamel, C., De Pauw, R.M., and Knox, R.E. 2012. Genetic variability in arbuscular mycorrhizal
357 fungi compatibility supports the selection of durum wheat genotypes for enhancing soil ecological services
358 and cropping systems in Canada. Can. J. Microbiol.58: 293-302 (2012).
359 Smith, S.E., and Read, D.J. 2008. Mycorrhizal Symbiosis. Academic Press, Inc., San Diego, CA .
360 Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms
361 from cellular to ecosystem scales. Annu Rev Plant Biol. 63:227–250.
362 Spagnoletti, F.N., Fernandez, D.P.A., Tobar, G.N., and Chiocchio, V.M. 2012. Arbuscular mycorrhizae and
363 Rhizobium: a dual symbiosis of interest. Revista Argentina de microbiologia 45(2), 131-132.
364 Tawaraya, K. 2003. Arbuscular mycorrhizal dependency of different plant species and cultivars. Soil Sci
365 Plant Nutr. 49(5), 655-668.
366 Varma, A.K. 1979. Vesicular-arbuscular mycorrhiza and nodulation in soybean. Folia microbial. 24(6), 501-
367 502.
368 Walker, C., Mize, W., and McNabb, H.S. 1982. Populations of endogonaceous fungi at two populations in
369 central Iowa. Can J Bot. 60:2518-2529.
370 Zhu, Y.G., Smith, S.E., Barritt, A.R., and Smith, F.A. 2001. Phosphorus (P) efficiencies and mycorrhizal
371 responsiveness of old and modern wheat cultivars. Plant Soil 237(2), 249-255.
372 Zhu, X., Song, F., and Liu, S. 2011. Arbuscular mycorrhiza impacts on drought stress of maize plants by lipid
373 peroxidation, proline content and activity of antioxidant system. J Food Agric Environ. 9:583–587.

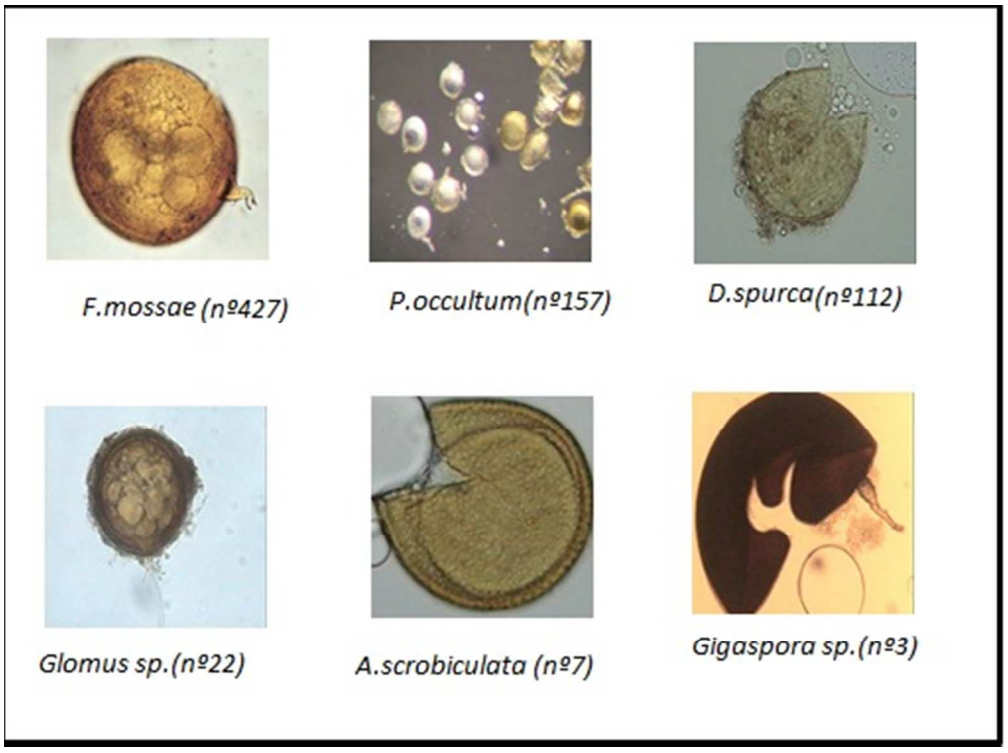

Fig. 1: Taxonomic identification of AMF spores of a mixed native AMF inoculum, isolated from soybean roots under a monoculture system.
134x99mm (96 x 96 DPI)

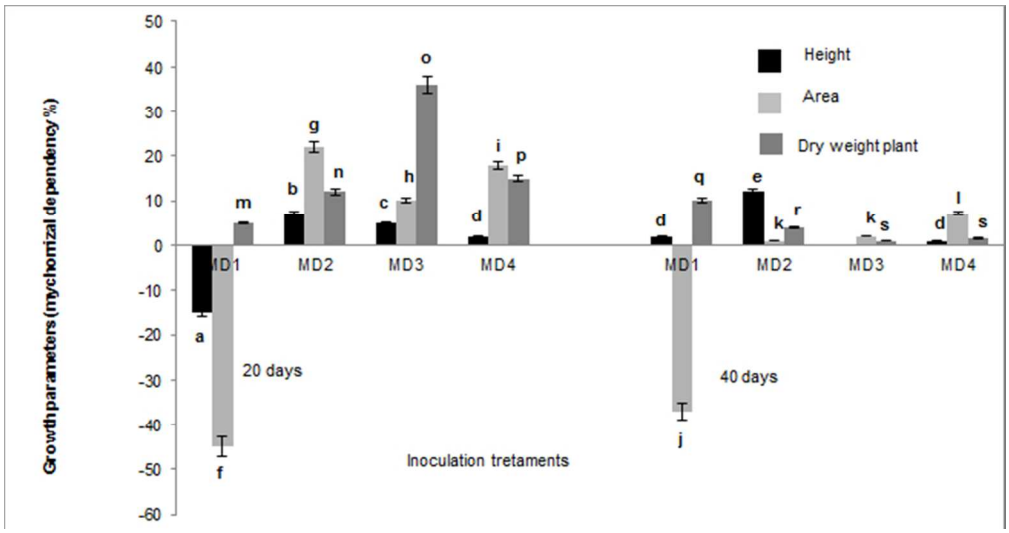

Fig. 2: Effects of inoculation with a mixed native AMF inoculum on growth parameters in soybean plants. MD: mycorrhyzal dependency; 1 and 2: improved genotypes; 3 and 4: unimproved genotypes. Results were obtained at 20 and 40 days under well-watered conditions.Treatments labeled with different letters indicate significant differences ($p<0.05$).
172x90mm (96 x 96 DPI)

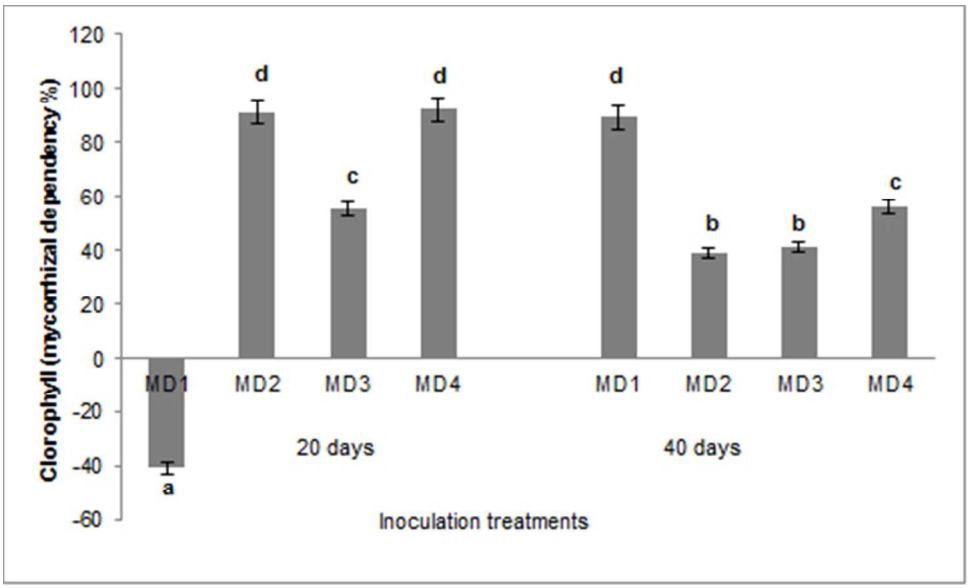

Fig. 3: Effects of inoculation with a mixed native AMF inoculum on total chlorophyll concentration in soybean plants. MD: mycorrhyzal dependency; 1 and 2: improved genotypes; 3 and 4: unimproved genotypes. Results were obtained at 20 and 40 days under well-watered conditions.Treatments labeled with different letters indicate significant differences ($p<0.05$).
127x76mm (96 x 96 DPI)

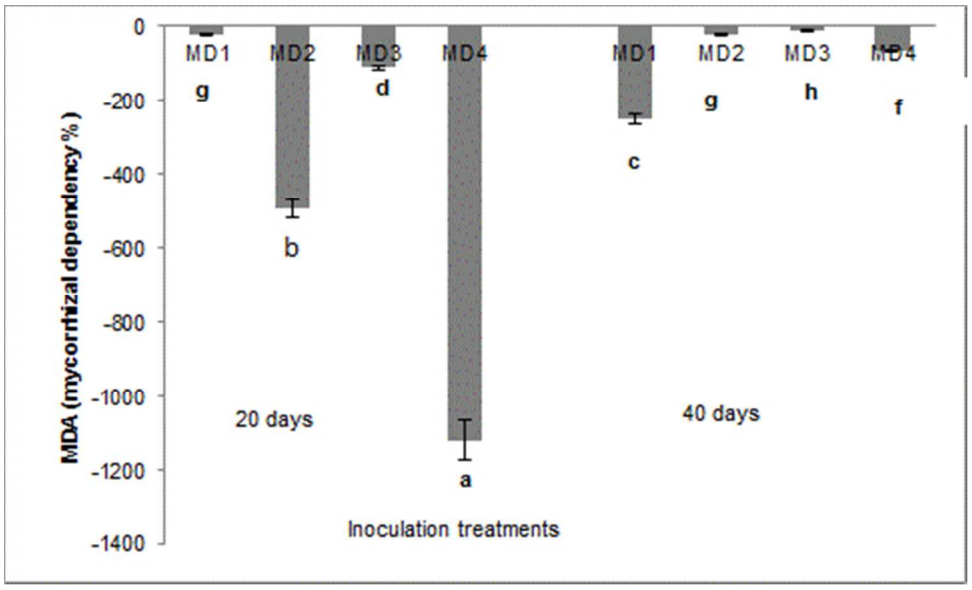

Fig. 4: Effects of inoculation with a mixed native AMF inoculum on antioxidant activity in soybean plants. MD: mycorrhyzal dependency; 1 and 2: improved genotypes; 3 and 4: unimproved genotypes. Results were obtained at 20 and 40 days under well-watered conditions.Treatments labeled with different letters indicate significant differences ($p<0.05$).
127x76mm (96 x 96 DPI)

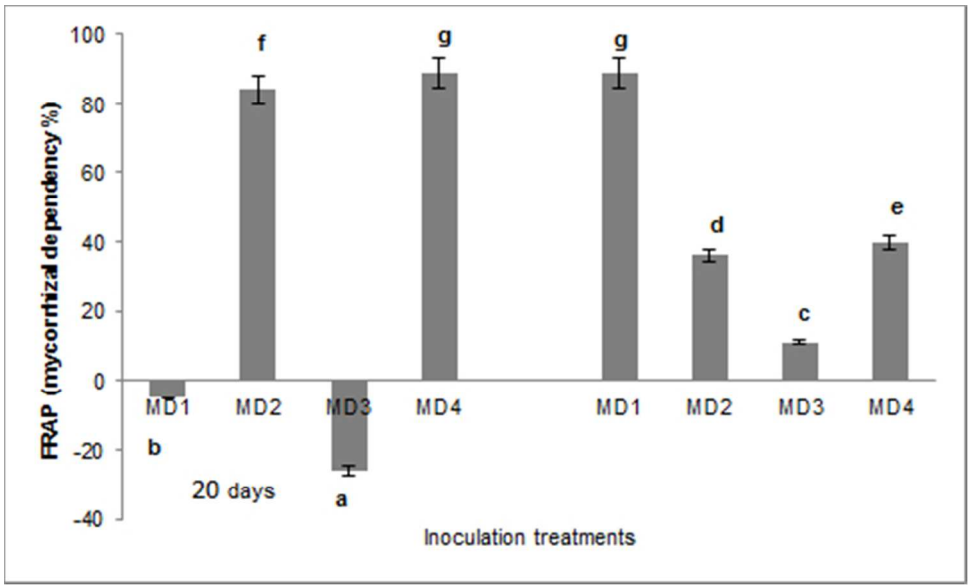

Fig. 5: Effects of inoculation with a mixed native AMF inoculum on Malondialdehyde concentration in soybean plants. MD: mycorrhyzal dependency; 1 and 2: improved genotypes; 3 and 4: unimproved genotypes. Results were obtained at 20 and 40 days under well-watered conditions.Treatments labeled with different letters indicate significant differences ($p<0.05$).
127x76mm (96 x 96 DPI)

Table 1. Arbuscular mycorrhizal fungus (AMF) colonization of roots in improved and unimproved soybean genotypes. (1 and 2: improved genotypes, 3 and 4: unimproved genotypes). Results were obtained at 20 and 40 days under well-watered conditions.

Genotypes	Time (days)	Root colonization (%)	Root colonization (%) by hyphae	Root colonization (%) by vesicles	Root colonization (%) by arbuscules
1	20	36 a	88 a	12 a	-
	40	38 b	73 b	27 b	-
2	20	22 c	60 c	4 c	36 a
	40	42 d	56 d	4 c	40 b
3	20	39 b	24 e	18 d	58 c
	40	64 f	21 f	16 e	63 d
4	20	22 c	51 g	3 f	46 e
	40	72 g	40 h	3 f	57 c

The same letter within each column indicates no significant difference among treatments ($p<0.05$).

Table 2. Number and total weight of nodules in improved and unimproved soybean genotypes. NM (non-mycorrhizal plants); M (mycorrhizal plants); 1 and 2: improved genotypes, 3 and 4: unimproved genotypes. Results were obtained at 40 days under well-watered conditions.

Genotypes	**N° of nodules/plant**	**Total Weight (g)**
1 NM	12 a	0,6 a
1 M	18 b	1,00 a
2 NM	13 a	0,6 a
2 M	23 c	1,3 a
3 NM	17 d	0,8 a
3 M	28 e	2,1 b
4 NM	18 d	0,8 a
4 M	30 e	2,3 b

The same letter within each column indicates no significant difference among treatments ($p<0.05$).